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CHAPTER 17

Probiotics against Digestive Tract Viral Infections

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ABBREVIATIONS

AdV Enteric adenovirus
AV Astrovirus
EV Enterovirus
NV Norovirus
ROS Reactive oxygen species
RV Rotavirus
TLR Toll-like receptor

1. INTRODUCTION

Gastrointestinal viral pathogens have great social and economic impact in both developed and developing nations. Gastrointestinal viruses are shed with human fecal wastes and are transmitted through the oro-fecal route by direct contact with an infected person and by consumption of, or contact with, contaminated water or food (Buesa and Rodríguez-Díaz, 2006; Knipe and Howley, 2007). Intestinal viral infections usually result in diarrhea of varying degrees, and oral or parenteral rehydration therapy is the most common treatment. However, there is a growing interest in the use of probiotic antiviral therapies due to their positive influence on human health. Probiotics are defined as live microorganisms that, upon ingestion in certain quantities, exert beneficial effects on the host. The most common probiotics are members of the lactic acid bacteria (*Lactobacillus*, *Streptococcus*, *Enterococcus*, etc.), *Bifidobacteria*, and some yeast strains. These microorganisms are generally recognized as safe, have a long history of use in food production, and are normal inhabitants of the gastrointestinal tract. This ecosystem is colonized by a diverse microbiota, which at some locations can reach up to 10^{12} microorganisms per gram of content, and it is constituted by approximately 1000 different species, making it one of the most dense and complex microbial ecosystems. The intestinal microbiota plays an important role in the organism's physiology and helps maintain host health (Collado et al., 2009). Thus, imbalances in its composition (dysbiosis) underlie some pathologies (e.g., inflammatory

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bowel diseases). The gut-associated immune system displays a hyporesponsiveness to this resident microbiota, but it is sensitive to products derived from it, the so-called microbial-associated molecular patterns (DNA, components of the cell wall, proteins, etc.), which are sensed by a complex family of receptors present in epithelial and immunocompetent cells (e.g., the Toll-like receptors – TLRs). A cross-talk is established between the microbiota and epithelial/immune cells, which influences cell proliferation and maturation and helps maintain the immune homeostasis and gut barrier functions (Gill and Prasad, 2008). The epithelium and the intestinal microbiota constitute a synergic physical and chemical defense line against pathogens. These defense functions are susceptible to being modulated by the use of probiotics.

There are many health benefits attributed to probiotics (prevention and treatment of intestinal infections, prevention and management of allergic diseases, enhancement of immune function, anticancer effects, cholesterol lowering, etc.), but the accumulated clinical data point to the treatment and prevention of infectious diarrhea as one of the health effects supported by sound scientific evidence. Besides their antibacterial activities, many studies have demonstrated that specific probiotics reduce the risk and shorten the duration of diarrheas associated with viral infections, especially in infants and children.

2. VIRUSES THAT INFECT THE GASTROINTESTINAL TRACT

Several types of viruses are able to replicate in the intestinal epithelium, but not all of them cause gastroenteritis (Buesa and Rodríguez-Díaz, 2006; Knipe and Howley, 2007). In the following sections, the most important viral groups responsible for gastrointestinal infections worldwide are described.

2.1 Noroviruses

Noroviruses (NVs) are members of the *Caliciviridae* family that infect the small intestine and cause the majority of foodborne and waterborne outbreaks of acute gastroenteritis worldwide. NVs contain a linear positive-sense single-stranded RNA genome. They have a high genetic variability, being classified in five genogroups (GI–GV) that are subdivided into genotypes. The major NVs infecting humans belong to the GI and GII genogroups, with the GII4 genotype emerging as the main genotype causing gastroenteritis outbreaks worldwide. The incubation time ranges from 15 to 48 h, leading to gastroenteritis for 12–60 h from the beginning of the symptoms. NV infection usually courses as a self-limited diarrhea and is characterized by vomiting, but in special cases, it can lead to severe dehydration and death.

2.2 Rotaviruses

Rotaviruses (RVs) are the main etiological cause of severe gastroenteritis and infantile morbidity worldwide in children under 5 years, the age when most of the population

is seroconverted and thus less susceptible to RV infection. RVs also lead to high childhood mortality in developing countries, causing ~500 000 deaths per year as a result of dehydration and deficient medical care. In developed countries, RV diarrheas are responsible for a large number of hospitalizations. Although some RV vaccines have been developed during the last few years, more economic alternatives would be desirable, especially in developing countries. RVs are 70-nm icosahedral viruses that belong to the family *Reoviridae* and infect mature enterocytes. Seven RV serogroups (A–G) have been described based on the antigenicity of the capsid VP6 protein. Most human pathogens belong to groups A, B, and C. RVs of group A are the most important from a public health standpoint. The virus is composed of three protein shells, an outer capsid, an inner capsid, and an internal core that surround 11 segments of double-stranded RNA. Three major structural and nonstructural proteins are of interest in epidemiological studies and vaccine development against group A RV: NSP4 (genotypes A–F), VP7 (G genotypes 1–15), and VP4 (P genotypes from 1 to 14). VP7 and VP4 are of special interest because they are able to elicit neutralizing antibodies.

2.3 Astroviruses

Astroviruses (AVs) are nonenveloped viruses with a positive-sense, single-stranded RNA genome. AV infections occur worldwide and their incidence ranges from 2% to 9% in both developed and developing countries. Outbreaks of AVs have been associated with consumption of sewage-polluted shellfish and ingestion of water from contaminated sources.

2.4 Enteric Adenoviruses

Enteric adenoviruses (AdVs) are nonenveloped, double-stranded DNA icosahedral viruses measuring 70–90 nm in diameter. AdVs are divided into two genera: *Mastadenovirus*, which includes viruses that infect mammals, and *Aviadenovirus*, which contains viruses that infect birds. In some countries, enteric AdVs (subtypes 40 and 41) are placed as the second etiologic agents of infantile gastroenteritis.

2.5 Enteroviruses

Enteroviruses (EVs) are named after their site of replication but rarely cause gastroenteritis, and the resulting infection is frequently asymptomatic or targets other organs. EVs belong to the family *Picornaviridae* and possess a positive-sense RNA genome. The two main representative EVs are polioviruses, causing poliomyelitis, and kuboviruses. Aichi virus, a member of the genus kubovirus, is responsible for gastroenteritis outbreaks usually caused by the consumption of contaminated oysters.

3. POSSIBLE MECHANISMS OF PROBIOTICS ACTION AGAINST INTESTINAL VIRUSES

The mechanisms for the antagonistic capacity of probiotics against microbial pathogens have been exhaustively investigated for microorganisms important in gastrointestinal infections, such as *Clostridium difficile*, *Helicobacter pylori*, *Salmonella*, or pathogenic *Escherichia coli*, where numerous *in vitro* and some clinical studies exist. The positive effects are attributed to multiple mechanisms (Servin, 2004), some of which can also be extended to viruses (summarized in Figure 17.1).

Probiotics are able to induce host cellular defenses against pathogenic bacteria, such as β-defensins synthesized by Paneth cells, and they also produce well-characterized antibacterial molecules such as organic acids, H_2O_2, or antimicrobial peptides (bacteriocins). Some authors have postulated that certain probiotics can produce antiviral substances (Botic et al., 2007; Seo et al., 2010), although their nature is unknown and *in vitro* viral

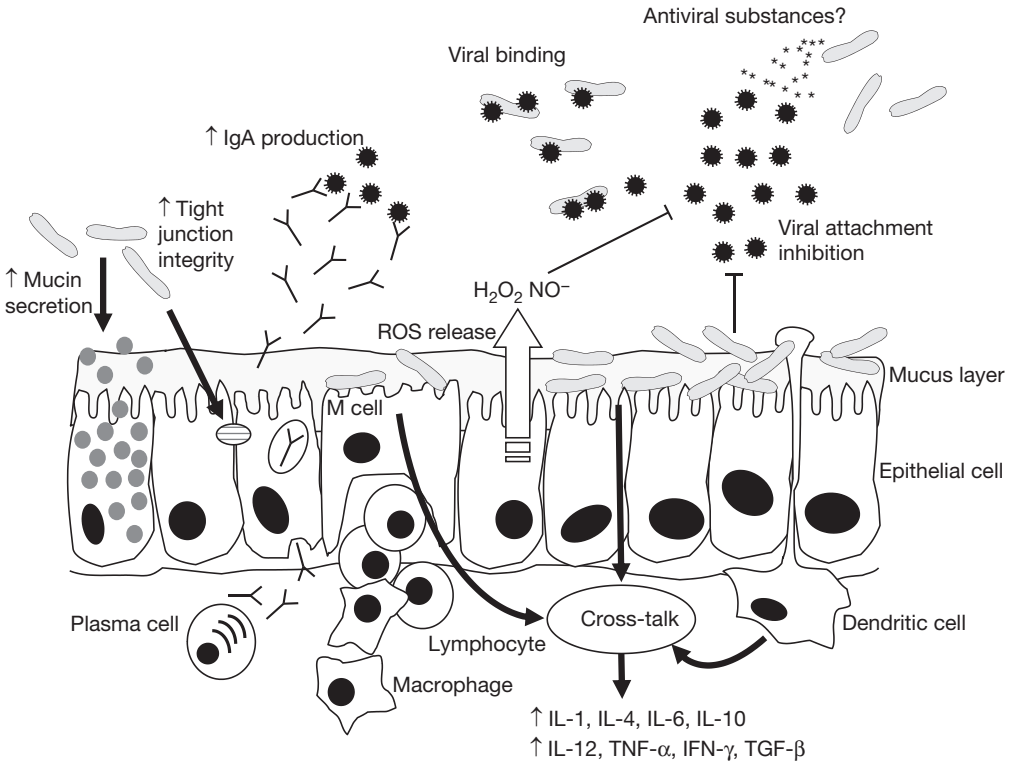

Figure 17.1 Proposed mechanisms for the antiviral effect of probiotics in gastrointestinal infections. Probiotics putatively interfere with viral replication at different levels, by blocking viral attachment, synthesizing antiviral compounds by itself, or inducing their synthesis by epithelial cells. The cross-talk established between probiotics and epithelial/immune cells enhances barrier functions and innate as well as adaptive immune responses.

inhibition with bacterial supernatants might be simply explained by the presence of organic acids.

One of the first viral infection mechanisms that can be targeted by probiotics is viral binding to host cells. Exclusion of pathogens by direct binding, attachment inhibition, or displacement has been thoroughly studied for bacterial pathogens in *in vitro* and *in vivo* studies using probiotics, but data on exclusion of viruses are scarce. Viruses can use oligosaccharides present as glycoconjugates on cellular surfaces as receptors for attachment and entry. RVs recognize sialic acid (*N*-acetylneuraminic acid) residues as a first step for cellular entry, whereas NVs display binding specificities toward α1,2-fucosylated carbohydrates and α2,3-sialylated carbohydrates, which form part of the histoblood group antigens expressed at mucosal surfaces. Many *Lactobacillus* and *Bifidobacterium* strains display lectin-like activities on their surfaces. Surface components from these bacteria have been characterized that bind to the highly glycosylated intestinal mucus and extracellular matrix proteins or are responsible for attachment to cultured enterocyte lines (e.g., Caco-2, HT-29, or T84 cell lines) (Lebeer et al., 2008). The surface layer proteins (SlpA) from *Lactobacillus* have been implicated in attachment to cellular surfaces and pathogen displacement, and other surface proteins with no evident secretion signals (e.g., chaperones and glycolytic enzymes) decorate the surface of *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus reuteri*, or *Lactobacillus johnsonii* strains and behave as sticky factors playing a role in adhesion. *Lactobacillus* species adapted to the gastrointestinal niche (*L. plantarum*, *Lactobacillus acidophilus*, *Lactobacillus gasseri*, *L. johnsonii*, and *L. reuteri*) possess surface-specialized proteins involved in mucin binding in a mannose-sensitive manner (lectin-like) or specific mucin-binding pili, as is the case for *Lactobacillus rhamnosus* GG. Other molecules of nonprotein nature present at the bacterial surface and reported to be involved in binding are lipoteichoic acids and exopolysaccharides. These types of molecules allow probiotics to attach to the intestinal mucosal surface and might be responsible for their persistence in this niche and, in addition, participate in viral exclusion and displacement from the surface of target cells. Besides, probiotic strains are able to directly bind viruses, which would promote their elimination in feces. This implies that some surface molecules (glycosylated proteins or other components of the cell wall) from probiotics could be mimicking viral receptors. Interestingly, the two strains of *L. rhamnosus* GG and *Bifidobacterium lactis* Bb-12 with a better-documented efficacy in infectious diarrhea exhibited the best binding ability to RV particles (Salminen et al., 2010).

Probiotics can modulate specific host pathways. They can induce the synthesis of molecules that interfere with some step of the viral cycle, increase the mucosal barrier function, or act as immunomodulators that enhance both innate and adaptive immune response.

Some studies have addressed the synthesis of reactive oxygen species (ROS) by cultured epithelial cells in the presence of probiotics. ROS can play defensive roles in the organism, and a correlation between ROS release induction and viral protection

for specific pairs of cultured cell lines/probiotic strains has been described (Maragkoudakis et al., 2010).

L. rhamnosus GG and *L. plantarum* 299v stimulate mucin secretion by upregulation of MUC-2 and MUC-3 genes in Caco-2 and HT-29 cells, respectively. Increased mucin secretion, which forms part of the epithelial mucus protective layer, may participate in viral exclusion by binding and entrapping viruses through specific mucin–viral interactions, promoting their shedding from the intestine and acting as a physical barrier that limits access to the epithelium.

Viral diarrheas involve varied mechanisms that result in deficient nutrient absorption or increased secretion of water and electrolytes. During infection, paracellular epithelial permeability can be increased and epithelial damage and apoptosis occur. *L. rhamnosus* GG and other members of the *L. casei/rhamnosus* group secrete to the culture medium-specific proteins (p40 and p75) that enhance barrier functions through mechanisms involving Akt and the PI-3K kinase and protect the intestinal epithelium from injury and apoptosis caused by inflammatory cytokines (tumor necrosis factor (TNF-α) and interferon gamma (IFN-γ)) or oxidative damage, maintaining the structure of the tight junctions and increasing the expression of specific proteins (e.g., zonula occludens-1, claudin, and occludin). Low-molecular-weight peptides produced by *L. rhamnosus* GG activate mitogen-activated protein kinases and induce cytoprotective heat-shock proteins HSP25 and HSP72 in intestinal cells. In general, probiotic strains maintain epithelial integrity and reduce the decrease in transepithelial resistance in cultures following pathogen infection. Thus, they may help to keep the intestinal barrier integrity which is compromised during viral infection.

In vitro and *in vivo* experiments have established that probiotics can modulate the synthesis of an array of cytokines, for example, interleukin (IL)-1, IL-2, IL-4, IL-6, IL-10, IL-12, IFN-γ, and TNF-α. This leads to a range of modulatory effects on immune cells: increased cytotoxic and phagocytic capacity of NK cells or macrophages and immune cell (T and B lymphocytes) proliferation and differentiation, which can result in increased antibody responses (Gill and Prasad, 2008). The consumption of fermented milk containing certain probiotics increased specific IgG and IgA titers when individuals were vaccinated against *Salmonella*, hepatitis B, influenza, or poliovirus. In this sense, *L. rhamnosus* GG was effective in promoting specific IgA-secreting cells and higher plasma IgA titers after RV infection (Kaila et al., 1992) and showed an adjuvant effect in RV vaccination.

4. LABORATORY EVIDENCE OF PROBIOTICS-CONFERRED RESISTANCE TO GASTROINTESTINAL VIRAL INFECTIONS

Most of the data on the effect of probiotics on viral gastrointestinal infections using *in vitro* and *in vivo* models have been obtained with RVs. This derives from the fact that, in

Table 17.1 Examples of the Efficacy of Probiotics against Gastrointestinal Viruses in Different *In Vitro* and *In Vivo* Models

Virus	Strains	Model	Effects	Reference
VSV	*B. breve* DSM 20091 *B. Longum* Q46 *L. paracasei* A14 *L. paracasei* F19 *L. paracasei* Q85 *L. plantarum* M1.1 *L. reuteri* DSM 12246	IPEC-J12 cell line	Reduced *in vitro* infection	Botic et al. (2007)
VSV	*L. paracasei* Q85 *L. paracasei* A14 *L. paracasei* F19 *B. longum* Q46	3D4/2 macrophage cell line	Increased antiviral response and decreased viral infection	Ivec et al. (2007)
RV	*L. acidophilus* NCFM *L. rhamnosus* GG	IPEC-J12 cell line	Protection and enhancement of innate immunity	Liu et al. (2010)
RV and TGEV	*L. rhamnosus* GG *L. casei* Shirota *E. faecium* PCK38 *L. fermentum* ACA-DC179 *L. pentosus* PCA227 *L. plantarum* PCA236 etc.	Six different cell lines (from human, pig, and goat)	Reduced *in vitro* infection	Maragkoudakis et al. (2010)
RV	*L. reuteri* Probio-16	TF-104 cell line	Reduced *in vitro* infection	Seo et al. (2010)
RV	*L. plantarum* 299v	Bovine intestinal epithelial cell line	Reduced infection and enhancement of innate immunity	Thompson et al. (2010)
RV	*B. bifidum*	Mice	Diarrhea reduction	Duffy et al. (1994)
RV	*L. casei* DN-114 001	Mice	Protection against infection and diarrhea	Guerin-Danan et al. (2001)
RV	*L. rhamnosus* GG	Suckling rats	Reduction of intestinal permeability after infection	Isolauri et al. (1993)
RV	*L. rhamnosus* GG	Mice	Treatment of diarrhea	Pant et al. (2007)
RV	*B. lactis* HN019	Piglets	Reduction in weaning diarrhea	Shu et al. (2001)
RV	*L. acidophilus* NCFM *L. reuteri* ATCC23272	Neonatal gnotobiotic piglets	Different TLR2, TLR3, and TLR9 patterns in antigen-presenting cells	Wen et al. (2009)

Continued

Table 17.1 Examples of the Efficacy of Probiotics against Gastrointestinal Viruses in Different *In Vitro* and *In Vivo* Models—cont'd

Virus	Strains	Model	Effects	Reference
RV	*L. acidophilus* NCFM *L. reuteri* ATCC23272	Neonatal gnotobiotic piglets	Neonatal B cell response	Zhang et al. (2008a)
RV	*L. acidophilus* NCFM *L. reuteri* ATCC23272	Neonatal gnotobiotic piglets	Differential distribution of monocyte, macrophages, and dendritic cells in ileum, spleen, and blood	Zhang et al. (2008b)

RV, rotavirus; TGEV, swine transmissible gastroenteritis coronavirus; VSV, vesicular stomatitis virus.

contrast to other gastrointestinal viruses (e.g., NVs), appropriate cell lines and animal models allowing infection and viral propagation are available. Table 17.1 summarizes the most relevant data on protection conferred by probiotics using different strains, experimental models (mice, rat, piglet, and cultured cells), and gastrointestinal viruses.

4.1 Animal Models

The utilization of animal models has shed some light into the mechanisms implicated in the beneficial effects of probiotics to counteract gastrointestinal viral infections. In addition, they provide tools for screening the viral protection conferred by different probiotic strains. The first relevant data on the positive effect of probiotic bacteria using an animal model were obtained from a model of suckling rats and group B RV strain IDIR (Isolauri et al., 1993). This work showed that a diet supplemented with *L. rhamnosus* GG decreased the jejunal permeability to macromolecules produced during RV infection, thus providing the first evidence of a protective mechanism of this probiotic against RV.

Milk fermented with *Bifidobacterium bifidum* and *L. casei* DN-114 001 was assayed for its effects in mice and germ-free suckling rats, respectively, demonstrating that survival and colonization of the digestive tract by the probiotics was linked to reduced diarrhea and viral shedding after infection with group A RV (Duffy et al., 1994; Guerin-Danan et al., 2001). The murine model has also been utilized to assess the effectiveness of *L. rhamnosus* GG combined with IgA antibodies against RV. This combination resulted in an effective prophylaxis against RV diarrhea, reducing the virus load in the intestines, preventing histopathological changes, and significantly reducing the diarrhea outcome measures (Pant et al., 2007).

The piglet model has also been employed to study the efficacy of probiotic treatment as well as to investigate the mechanisms of the conferred protection. *B. lactis* HN019 reduced the diarrhea associated to RV in a piglet model, showing the potential use of probiotics in farms to reduce the severity of the weanling diarrhea and to improve the feed conversion efficiency associated to a reduction of RV in feces and increased

intestinal pathogen-specific antibody titers. This work postulates the enhanced immune-mediated response as the possible mechanism for the beneficial effect of this probiotic (Shu et al., 2001). Following this postulation, studies on the virus-specific B and T cell responses induced by the attenuated and virulent Wa human RV strains in gnotobiotic piglets, with or without *L. acidophilus* or *L. reuteri* colonization, demonstrated an enhanced immunity against RV conferred by the probiotics (Zhang et al., 2008a). These studies were completed by using *L. acidophilus* and *L. reuteri* and the virulent human Wa RV strain to assay the distribution and maturation of antigen-presenting cells. The authors showed a differential distribution and frequency of monocytes/macrophages and dendritic cells. Furthermore, differences in the maturation marker CD14 and a differential activation of TLRs and innate immunity cytokines IFN-γ and IL-4 were observed, identifying the enhanced immune response as a possible mechanism of viral protection (Wen et al., 2009; Zhang et al., 2008b).

4.2 Cell Culture Models

A cell culture system was reported to provide evidence on viral protection conferred by probiotic strains by using nontumorigenic porcine intestinal epithelial cells (IPEC-J12) and alveolar macrophages (3D4/2). This study aimed to elucidate the protection ability and seek for a mechanistic explanation of the effect of several probiotics against a model virus (vesicular stomatitis virus or VSV) (Botic et al., 2007). When seven different probiotic strains (*Bifidobacterium breve* DSM 2009, *Bifidobacterium longum* Q46, *Lactobacillus paracasei* A14, *L. paracasei* F19, *L. paracasei/rhamnosus* Q85, *L. plantarum* M1.1, and *L. reuteri* DSM 12246) were analyzed, the protection conferred was up to 70%, and all strains prevented VSV cellular binding when the cell monolayers were preincubated with the strains for 24–48 h prior to the viral challenge. Furthermore, previous adsorption of VSV directly to probiotics had the same effect, giving a mechanistic explanation for the observed protection. Culture supernatants of probiotics were assayed to elucidate if they were able to produce any antiviral metabolite. Three strains, *B. longum* Q46, *L. plantarum* M1.1, and *L. reuteri* DSM 12246, produced metabolites able to reduce the viral infection by up to 67% (Botic et al., 2007). Similar results have been obtained with culture supernatants of the *L. reuteri* strain Probio-16, isolated from pig feces, which blocked viral infection of a group A porcine RV in an African green monkey epithelial cell line (Seo et al., 2010).

Ivec and collaborators showed that four of the strains previously assayed in the Botic's study (*L. paracasei/rhamnosus* Q85, *L. paracasei* A14, *L. paracasei* F19, and *B. longum* Q46) were able to reduce viral infectivity in macrophages (3D4/21), increasing the antiviral response against VSV measured as IFN-γ, IL-6, and nitric oxide production and thus confirming a cross-talk between probiotics and macrophages (Ivec et al., 2007).

The same cell model (IPEC-J12) has been used to study the influence of probiotics on the innate immune response to RV. Two probiotic strains with known anti-rotaviral

effect were utilized (*L. acidophilus* NCFM and *L rhamnosus* GG), but none of them were able to protect the cell cultures against porcine RV OSU strain infection. *L. acidophilus* was able to increase the IL-6 response, which was consistent with the adjuvant effect of *L. acidophilus*. On the contrary, *L. rhamnosus* GG was able to increase the expression of TLR-2 and decrease the levels of IL-6, confirming an anti-inflammatory effect (Liu et al., 2010). In this line of experiments, priming of bovine intestinal epithelial cells with *L. plantarum* 299v prior RV infection resulted in upregulation of genes involved in innate immunity (TLR-3, TLR-7, TLR-9, IFN-α, and IFN-β) and a decrease in virus infection (Thompson et al., 2010).

In general, the attempts to use cell culture models to find common mechanisms that confer viral resistance have failed, providing evidence that protection is conferred by a multifactorial mechanism that depends on viral agent, cell line utilized, and the probiotic strain (Maragkoudakis et al., 2010).

5. CLINICAL EVIDENCE

Due to their worldwide prevalence, and as already mentioned for the laboratory evidences, most of the clinical studies on the effect of probiotics on gastrointestinal viruses have been focused on the incidence of RV acute diarrhea in children. However, it has to be noted that the diverse etiology makes it difficult to assign an episode of infectious diarrhea to a particular pathogen without proper serological, microbiological, or molecular studies. A number of randomized placebo-controlled trials have demonstrated that oral intake of certain strains, particularly *L. rhamnosus* GG and *B. lactis* Bb-12, promotes a faster recovery from acute RV diarrhea (shortening of diarrhea by 1–1.5 days) and reduced symptom severity (Table 17.2). Moreover, probiotics are well tolerated and no adverse effects are generally reported. These positive results have also been documented for these and other probiotics in infectious diarrhea of non-RV origin or antibiotic-associated diarrhea, supporting the use of probiotics as an adjuvant therapy combined with classical rehydration. However, studies on the prophylactic use of probiotics to reduce the risk of diarrhea in children at day care centers or nosocomial diarrheas originated by RV have produced modest evidences or conflicting results. As an example, studies conducted on 81 hospitalized children orally given two daily doses of 6×10^9 *L. rhamnosus* GG bacterial cells resulted in an 87% reduction in the incidence of RV diarrhea (Szajewska et al., 2001). On the contrary, a daily intake of 10^{10} bacteria of the same strain in 220 children did not show an effect in the risk of RV diarrhea compared with placebo (Mastretta et al., 2002).

Unfortunately, most of the clinical data are merely descriptive and do not provide a mechanistic explanation for the conferred benefits or a clear confirmation of the diarrhea etiology. Only in a few examples, reduced viral shedding in feces (Fang et al., 2009; Mao et al., 2008; Rosenfeldt et al., 2002) and increased immune response (IgA levels) have

Table 17.2 Examples of the Efficacy of Probiotics on RV Diarrhea in Humans

Type of analysis	Strains	No. of subjects	Effects	Reference
DRCT	VSL#3 (mix of eight probiotic strains)	224	Reduced stool frequency and improved stool consistency	Dubey et al. (2008)
DRCT	*L. acidophilus* and *B. infantis*	100	Reduction in diarrhea duration in RV-positive and negative groups	Lee et al. (2001)
DRCT	Bifilac (probiotic mix)	80	Reduced stool frequency and diarrhea duration	(Narayanappa, 2008)
RCT	*L. rhamnosus* 19070-2 and *L. reuteri* DSM 12246	69	Reduced frequency of RV antigen in feces in treated group versus control	Rosenfeldt et al. (2002)
SRCT	*L. acidophilus*, *L. rhamnosus*, *B. longum*, and *Saccharomyces boulardii*	75	Reduced diarrhea duration	Teran et al. (2009)
RCT	*L. rhamnosus* GG	71	Reduced diarrhea duration	Isolauri et al. (1991)
RCT	*L. rhamnosus* 35	23	Dose–response effect in reduction of RV shedding	Fang et al. (2009)
RCT	*L. rhamnosus* GG	39	Reduced diarrhea duration	Kaila et al. (1992)
DRCT	*L. rhamnosus* GG	220	No effect on preventing nosocomial RV infection	Mastretta et al. (2002)
RCT	*L. rhamnosus* GG	123	Reduction in RV diarrhea duration, no effect on bacteria-induced diarrhea	Shornikova et al. (1997a)
DRCT	*L. rhamnosus* (three strains)	39	Reduction in diarrhea duration, no effect on non-RV diarrhea	Szymanski et al. (2006)
DRCT	*L. rhamnosus* GG	81	Reduced risk of RV nosocomial diarrhea	Szajewska et al. (2001)
RCT	*B. lactis* Bb-12	224	No influence in diarrhea duration, decrease in RV shedding	Mao et al. (2008)
DRCT	*B. lactis* Bb-12	55	Reduced incidence of diarrhea and RV shedding	Saavedra et al. (1994)
DRCT	*L. paracasei* ST11	230	No effect in RV-induced diarrhea	Sarker et al. (2005)
RCT	*L. reuteri* SD 2112	40	Reduced diarrhea duration	Shornikova et al. (1997b)

RCT, randomized placebo-controlled trial; DRCT, double-blinded, randomized placebo-controlled trial; SRCT, single-blinded, randomized placebo-controlled trial.

been shown (Kaila et al., 1992). The fact that probiotics do not generally colonize the gastrointestinal tract and persist in it only transiently suggests that the benefits are evidenced only during the probiotic supplementation phase, with no sustained protection. Therefore, a daily intake of a high amount of cells, more than 10^9 viable cells in most cases, is needed to reach the required minimal effective doses.

6. CONCLUSIONS AND PERSPECTIVES

In vitro, *in vivo*, and clinical trials have provided conclusive results on the efficacy of probiotics against intestinal viruses. Treatment of acute diarrhea in children seems the most justified area of application, although the efficacy of probiotics in gastrointestinal viral infections in adults is still questionable. Research in the field of probiotics and their application needs, however, to address several key issues: (i) The heterogeneity of the experimental designs, the number of patients, the analyses, and the diarrhea outcome measurements, which make evaluation of different clinical trials very difficult. This warrants the design of more standardized trials with higher numbers of subjects in order to obtain better-supported conclusions. This will help to define better treatment protocols with established doses, frequencies, and specific strains. (ii) The research has to be extended to other viruses different from RVs that are important agents causing gastroenteritis, such as NVs. For this, new infection models are needed and an adequate identification of the infectious agent (viral or bacterial nature) in clinical trials is required. (iii) The reported effects are strain specific and proven only for a few probiotics. *L. rhamnosus* GG, the strain for which a higher number of clinical trials have been conducted, has been shown to be effective against RV but it did not have any effects on enteropathogenic bacteria. On the contrary, other strains have shown effects on diarrheas of viral and nonviral origin. Collections of probiotics need to be screened for their antiviral effects so that specific strains able to inhibit different viruses can be identified. The availability of several *in vitro* (cell lines) and animal models of infection will help in this screening and in the detailed investigation of the molecular mechanisms and probiotic-derived compounds mediating the effects. Although immunomodulation emerges as the main plausible explanation for the antiviral effects, the underlying mechanisms are far from being understood and constitute an interesting research field for the future.

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